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**Individual differences in cortisol stress response predict increases in voice pitch during  
exam stress**

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**ACCEPTED MANUSCRIPT**

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## **Abstract**

Despite a long history of empirical research, the potential vocal markers of stress remain unclear. Previous studies examining speech under stress most consistently report an increase in voice pitch (the acoustic correlate of fundamental frequency,  $F0$ ), however numerous studies have failed to replicate this finding. In the present study we tested the prediction that these inconsistencies are tied to variation in the severity of the stress response, wherein voice changes may be observed predominantly among individuals who show a cortisol stress response (i.e., an increase in free cortisol levels) above a critical threshold. Voice recordings and saliva samples were collected from university psychology students at baseline and immediately prior to an oral examination. Voice recordings included both read and spontaneous speech, from which we measured mean, minimum, maximum, and the standard deviation in  $F0$ . We observed an increase in mean and minimum  $F0$  under stress in both read and spontaneous speech, whereas maximum  $F0$  and its standard deviation showed no systematic changes under stress. Our results confirmed that free cortisol levels increased by an average of 74% (ranging from 0-270%) under stress. Critically, increases in cortisol concentrations significantly predicted increases in mean  $F0$  under stress for both speech types, but did not predict variation in  $F0$  at baseline. On average, stress-induced increases in voice pitch occurred only when free cortisol levels more than doubled their baseline concentrations. Our results suggest that researchers examining speech under stress should control for individual differences in the magnitude of the stress response.

**Keywords:** speech under stress; fundamental frequency; cortisol; psychological stress; exam stress

## **Highlights**

- The finding that voice pitch increases under stress is often not replicated
- We tested whether this is tied to variation in the severity of the stress response
- Voice recordings and saliva were collected at baseline and during exam stress
- Increases in cortisol levels predicted voice pitch under stress but not at baseline
- Researchers examining speech under stress should control for the stress response

## Introduction

Studies examining the acoustic correlates of stress have produced widely mixed results (reviewed in Giddens et al., 2013; Kirchhübel et al., 2011). In large part these inconsistencies stem from differences in how stress is defined (Murray et al., 1996), difficulties related to inducing and measuring genuine stress responses in the laboratory (Berger et al., 1987; Kudielka and Kirschbaum, 2005; Ruiz et al., 1996; Scherer, 1979; Tolkmitt and Scherer, 1986), and substantial variation in the magnitude of the stress response across individuals (Berger et al., 1987; Johannes et al., 2000; Kudielka et al., 2007; Streeter et al., 1983). Exam stress, the most significant source of stress experienced by students, peaking just prior to an academic examination (Robotham, 2008), offers an ecologically valid context in which to examine the effects of psychological stress on the voice. Psychological stress increases the activity of the hypothalamic adrenal (HPA) axis and circulating stress hormone levels, particularly cortisol (Dickerson and Kemeny, 2004; Kudielka and Kirschbaum, 2005), and is known to have multiple and apparent effects on the body including increased breathing rate, muscle tension, and changes in salivation rate, that may in turn affect vocal production (Kirchhübel et al., 2011; Scherer, 1981, 1979).

Voice pitch (the acoustic correlate of fundamental frequency,  $F_0$ ) is inversely related to the rate of vocal fold vibration, and thus increases as the vocal folds stretch and become tenser or when sub-glottal pressure and vocal intensity increase (Hollien, 2014; Titze, 2011), as often occurs under psychological stress. Indeed, an increase in voice pitch is the longest and most commonly reported finding in previous studies examining speech under stress (reviewed in Giddens et al., 2013; Kirchhübel et al., 2011). However, many studies have failed to replicate this finding (e.g., Dietrich and Abbott, 2012; Hecker et al., 1968; Johannes et al., 2000; Streeter

et al., 1983; Tolkmitt and Scherer, 1986; Van Lierde et al., 2009). Others report an increase in minimum voice pitch or a decrease in its standard deviation ( $F0\ sd$ ), with no systematic change in mean pitch (Park et al., 2011; Tolkmitt and Scherer, 1986). Previous studies also consistently indicate that voice pitch increases more in natural than artificially induced stress scenarios (Kirchhübel et al., 2011; Ruiz et al., 1996). It is possible that there are no reliable acoustic markers of psychological stress (Streeter et al., 1983). Alternatively, as has been suggested by many researchers, these inconsistencies in previous work may be tied to variation in the severity of the stress response, wherein vocal changes may be observed only in particular contexts (e.g., natural stress scenarios) or among individuals surpassing a critical threshold of stress (Beatty and Behnke, 1991; Johannes et al., 2000; Kirchhübel et al., 2011; Scherer, 1979). Despite this parsimonious possibility, only a handful of studies examining speech under stress have controlled for individual differences in stress levels (Hecker et al., 1968; Sigmund, 2006) or in participants' propensity toward anxiety (Beatty and Behnke, 1991; Tolkmitt and Scherer, 1986).

The aim of the present study was to test whether individual differences in the stress response, quantified by free cortisol levels measured from saliva, predict the magnitude of voice pitch changes under stress. To test this prediction we collected voice recordings and saliva samples from undergraduate psychology students two weeks prior to (baseline) and immediately prior to (stress) an academic oral examination, and compared changes in free cortisol levels and voice pitch at both times. Voice recordings included both read and spontaneous speech from which we measured mean, minimum, maximum, and the standard deviation in fundamental frequency.

## **Methods & Materials**

### **Participants**

Thirty-four students took part in the study (aged 21-32,  $M = 22.7$ ,  $sd = 2.0$ , all female). All students were registered in an upper-level psychology course and completed an oral examination as a course requisite. Students who volunteered to take part in the study were informed that voice recordings and saliva samples would be collected approximately two weeks before and on the day of the oral examination, and that their choice to take part in the study would in no way affect their exam grade. Participants completed a prescreening questionnaire. On this basis, data from 3 participants were excluded from the study for one or more of the following reasons: the participant was taking steroids such as allergy medications containing corticosteroids, smoked more than 15 cigarettes per day, was pregnant or breastfeeding, and/or had a disease of the mouth or chronic illness (see Kudielka et al., 2007). All participants provided written informed consent to participate.

## **Procedure**

Voice recordings and saliva samples were collected from each participant in two separate sessions following a similar procedure (see Data Collection). Baseline sessions took place approximately two weeks ( $M = 12.3$ ,  $sd = 2.2$  days) before the students' oral examination was scheduled to take place. During the baseline session, each participant provided a voice recording, completed a demographic questionnaire, and provided a baseline saliva sample 15 minutes afterward. Stress sessions took place on the day of the oral exam. Upon arrival, participants blindly drew three exam questions out of a jar, and were given 10 minutes to prepare their oral responses to these questions. As previous studies indicate that exam stress peaks immediately before and declines during the exam (Gadzella et al., 1998; Mechanic, 1962), participants' voices were recorded immediately before the oral exam. Oral exams took place in a

private room with the instructor and lasted approximately 15 minutes, immediately after which each participant provided a saliva sample.

It takes approximately 15 minutes for the production of free cortisol by the adrenal glands to manifest itself in saliva following psychological stress (Kirschbaum et al., 1993), therefore saliva collection followed voice recording by 15 minutes. Cortisol responses to social stress are largely unaffected by time of day (Bouma et al., 2009; Kudielka et al., 2004b). However, all sessions were scheduled in the afternoon to control for the awakening cortisol response and diurnal fluctuations in cortisol levels (Edwards et al., 2001; Kudielka and Kirschbaum, 2003). Within participants, baseline and stress sessions were also scheduled within 0-90 minutes ( $M=19.4$ ,  $sd=40.1$  min) of the same time of day. Participants confirmed having not consumed any food, caffeine, nicotine, vitamins or medication, having not brushed their teeth, and having not engaged in any form of rigorous exercise within two hours of each session (Kudielka et al., 2007).

The study was performed in accordance with the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki) and was approved by the University of Wroclaw Institutional Review Board.

## **Data Collection**

### ***Voice recording***

In both baseline and stress sessions, voice recordings were conducted in the same quiet room using an M-Audio condenser microphone with a cardioid pick-up pattern and at a distance of 5-10 cm. Participants were asked to describe their studies (e.g., subject area or major, year of degree, and department). This constituted the spontaneous speech condition. Participants were



also asked to familiarize themselves with and subsequently read the first five sentences of the Rainbow Passage (Fairbanks, 1960; Polish translation, see Appendix). This constituted the read speech condition. Audio was digitally encoded using an M-Audio Fast Track ultra interface at a sampling rate of 44.1 kHz and 16-bit amplitude quantization and stored onto a computer as WAV files.

### ***Saliva collection***

Saliva samples were collected in duplicate or triplicate from each participant during each session into 2 ml polypropylene microtubes (SARSTEDT®) using the passive drool method (Gröschl, 2008). Sugar-free gum was used to stimulate saliva flow (Salimetrics LLC, State College, PA, USA). Saliva samples were immediately frozen at -25°C until being transported to the Institute of Genetics and Microbiology, where the samples were kept at a temperature of -70°C until analysis.

## **Data Analysis**

### ***Voice measurement***

Acoustic measurements were performed in Praat (Boersma and Weenink, 2015) while blind to speaker identity and session. For each voice recording (4 per participant), we measured voice pitch as mean fundamental frequency ( $F0$  mean), minimum fundamental frequency ( $F0$  min), maximum fundamental frequency ( $F0$  max), and the standard deviation in fundamental frequency ( $F0$  sd). All measures were taken from voiced speech segments only. Spontaneous speech recordings were analyzed in full (duration  $M=4.9$ ,  $sd=1.4$  s). To analyze a comparable duration of read speech, we selected the central sentence of the Rainbow Passage (see

Appendix). All  $F0$  parameters were measured using Praat's autocorrelation algorithm with a search range set to 100-600 Hz (Boersma and Weenink, 2015).

### ***Hormone measurement***

Hormone concentrations were measured at the Institute of Genetics and Microbiology. Prior to analysis, frozen saliva samples were brought to room temperature and then centrifuged for 10 minutes in order to separate mucins. Clear colorless supernatant from samples taken from the same participant and condition were transferred into a separate sampling device, mixed on an orbital shaker and used for testing. Active free cortisol levels were measured using enzyme-linked immunosorbent assay (ELISA) and commercial kits (DEMEDITEC®, Germany, cat. number DES6611). Mixed saliva samples were assayed in duplicate following the manufacturer's instructions. Concentrations were calculated in relation to the standard curve and are expressed in ng/ml. The average assay sensitivity was 0.014 ng/ml, and the intra-assay and inter-assay coefficients of variation were less than 5.9% and less than 9.4%, respectively.

## **Results**

### **Changes in Free Cortisol Under Stress**

Participants' free cortisol concentrations averaged 5.6 ng/ml in the baseline session (range: 0.8 – 16.3,  $sd = 3.2$  ng/ml) compared to 9.7 ng/ml in the stress session (range: 1.8 – 21.8,  $sd = 5.7$  ng/ml). Cortisol levels increased by an average of 74% under stress within participants, however, the magnitude of this stress response varied widely (ranging from 0 – 270 % increase). There were no significant differences between women using hormonal contraception ( $n=8$ ) and those who were not ( $n=26$ ) in measured cortisol concentrations at baseline ( $F_{1,33}=0.42$ ,  $p=.52$ ),

during stress ( $F_{1,33}=1.19, p=.28$ ), or in the change in cortisol concentrations from baseline to stress ( $F_{1,33}=2.4, p=.14$ ).

### Changes in Voice Pitch Under Stress

Voice pitch measures are summarized in Table 1.

Table 1. Means and standard deviations ( $M(sd)$ ,  $n = 34$ ) of voice pitch parameters at baseline and during stress, as well as the change from baseline to stress, in both spontaneous and read speech.

Voice Parameter	Spontaneous Speech			Read Speech		
	Baseline	Stress	Change <sup>a</sup>	Baseline	Stress	Change <sup>a</sup>
<i>F0</i> mean	219.9	224.4	4.9	220.5	222.4	1.9
(Hz)	(14.4)	(13.8)	(9.8)	(15.6)	(13.7)	(9.2)
<i>F0</i> min	142.5	155.0	12.5	147.0	160.2	13.2
(Hz)	(37.5)	(38.5)	(35.1)	(36.7)	(32.1)	(36.1)
<i>F0</i> max	347.8	370.3	22.5	301.6	303.6	2.0
(Hz)	(65.0)	(92.2)	(76.4)	(29.2)	(33.0)	(28.7)
<i>F0 sd</i>	33.2	35.62	2.4	31.4	28.1	-3.3
(Hz)	(8.7)	(11.7)	(10.3)	(12.2)	(12.2)	(9.0)

Abbreviations: *F0* = fundamental frequency; Hz = hertz.

*a.* Change in voice parameter under stress, i.e. the difference between sessions (stress – baseline).

To test for changes in voice pitch under stress, we conducted repeated measures ANOVAs separately for each voice pitch parameter. Each model included session (baseline, stress) and speech type (spontaneous, read) as within-subject variables. These results are given in Table 2. Mean *F0* and minimum *F0* did not differ between spontaneous and read speech, and for both of these speech types, mean *F0* and minimum *F0* increased significantly under stress.

Maximum  $F0$  and  $F0\ sd$  were generally higher in spontaneous than read speech, but showed no systematic changes under stress.

Table 2. ANOVA table examining voice changes under stress in spontaneous and read speech

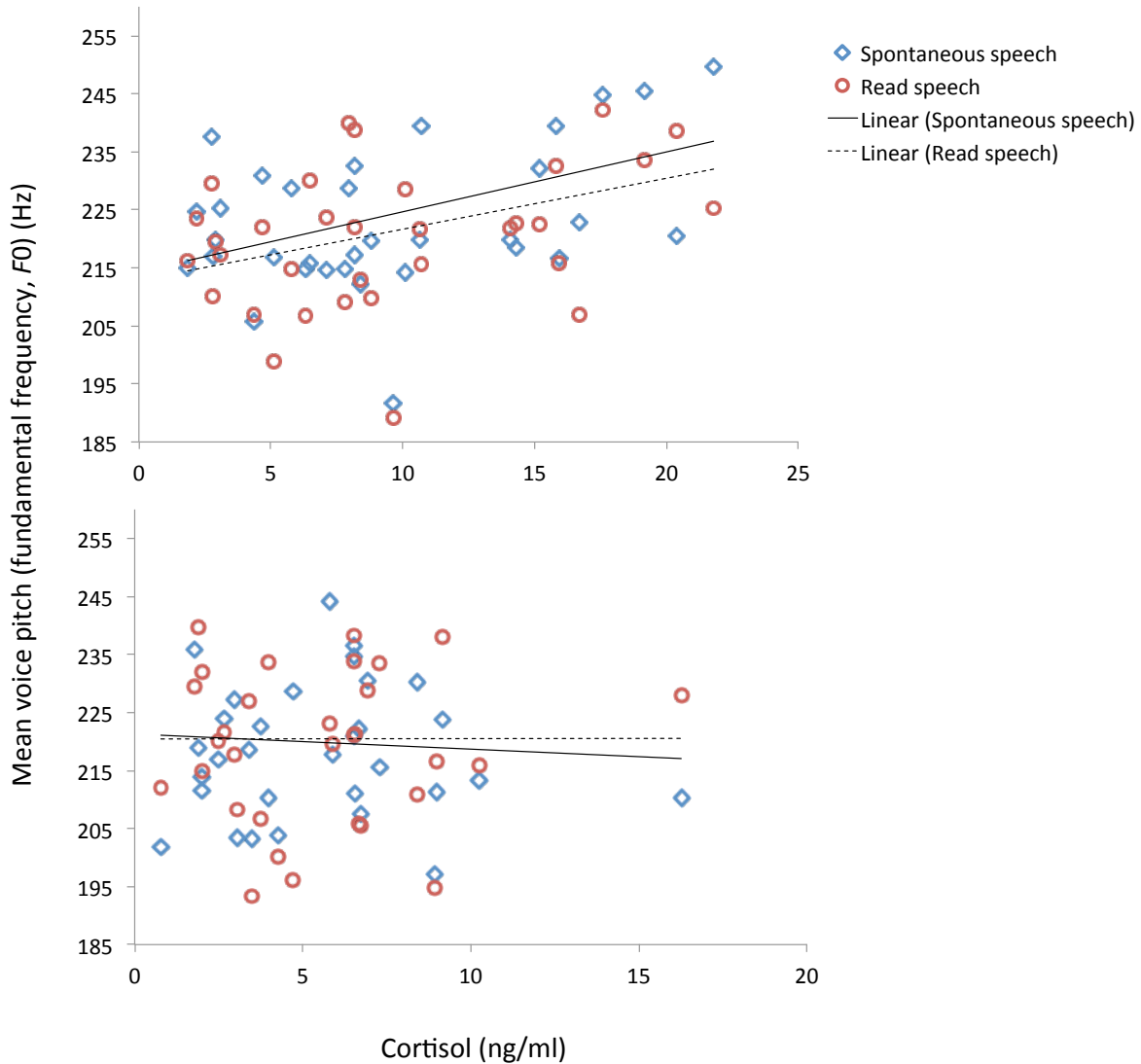
Voice Parameter	Session (Baseline or Stress)		Speech Type (Spontaneous or Read)		Condition x Speech Type	
	$F$	$p$	$F$	$p$	$F$	$p$
$F0$ mean	6.82	.014 *	0.59	0.449	2.32	.138
$F0$ min	4.92	.034 *	0.37	.545	0.03	.859
$F0$ max	2.50	.12	17.62	<.001 *	2.30	.139
$F0\ sd$	0.11	.747	5.81	.022 *	7.42	.011 *

$F$ -ratio ( $F$ ) and  $p$ -value ( $p$ ) for repeated measures ANOVA ( $df = 31$ ). Significant effects ( $p < .05$ ) are indicated with an asterisk.

### The Relationship between Changes in Free Cortisol and Voice Pitch

We tested whether individual differences in free cortisol concentrations predicted the observed changes in mean  $F0$  and minimum  $F0$  under stress. We used nonparametric Spearman's  $\rho$  ( $r_s$ ) correlations to examine relationships among these variables, as both cortisol concentrations and voice parameters were non-normally distributed (Shapiro-Wilk  $p < .05$ ). Participants' free cortisol levels significantly and positively predicted their mean  $F0$  in the stress session for both spontaneous speech ( $r_s = .46$ ,  $p = .015$ ) and read speech ( $r_s = .45$ ,  $p = .016$ ). The effect sizes of these relationships were fairly large (Cohen et al., 2003), wherein cortisol levels accounted for 20% of the variation in mean  $F0$  under stress, and followed a linear trajectory (Figure 1). Regression analyses indicated that for every unit increase in free cortisol concentrations (1 ng/ml), mean  $F0$  increased by 1.1 Hz. Hence, on average, voice pitch exceeded the mean baseline value (220 Hz) when cortisol levels were higher than 10 ng/ml (i.e.,

approximately twice the baseline cortisol level). This occurred in 37% of participants. Importantly, cortisol levels did not predict variation in mean  $F0$  in the baseline session (spontaneous speech:  $r_s = .10$ ,  $p = .60$ ; read speech:  $r_s = -.03$ ,  $p = .87$ ; see Figure 1), nor variation in minimum  $F0$  in either session (all  $r_s < .26$ , all  $p > .18$ ).



**Figure 1.** Mean voice pitch ( $F0$ ) increased with increases in free cortisol concentrations during stress for both spontaneous speech ( $r_s = .46$ ) and read speech ( $r_s = .45$ )(top panel), whereas cortisol did not predict mean voice pitch in the baseline session (bottom panel).

## Discussion

Research on speech under stress boasts a long history, yet the acoustic markers of stress remain unclear due to considerable inconsistencies in this body of work. Even the most consistently reported finding – that voice pitch increases under stress – is often not replicated and the reason for this remains unclear (Giddens et al., 2013; Kirchhübel et al., 2011). In the present study we tested the hypothesis that inconsistencies in past work may be tied to variation in the severity of the stress response, wherein voice changes may be observed predominantly among participants whose stress responses exceed a critical threshold.

Our results indicated that, on average, mean and minimum voice pitch increased among students immediately prior to completing an oral examination, whereas maximum pitch and variation in pitch did not change systemically under stress. Stress-induced increases in free cortisol levels and vocal parameters showed large individual differences. Indeed, free cortisol concentrations increased anywhere from 0-270% immediately preceding the oral exam compared to resting levels two weeks prior. Supporting our prediction, increases in cortisol strongly predicted increases in mean voice pitch, explaining 20% of the variation in voice pitch under stress. Voice pitch increased predominantly when cortisol levels more than doubled their baseline concentration, which occurred in less than half of our participants. Our results therefore suggest that, in order to reduce inconsistencies in future work, researchers examining speech under stress should either limit their acoustic analyses to the voices of verified stressed out individuals, or statistically control for individual differences in the stress response.

Exam stress constitutes a natural form of psychological stress and one that is known to strongly activate the HPA stress response in most people (Robotham, 2008). This may explain

why we observed a main effect of stress on voice pitch changes, even when having not controlled for severity in the stress response. However, it is important to highlight that this statistical main effect was small. On average, ignoring the severity of the stress response, mean  $F_0$  increased by only 5 Hz and minimum  $F_0$  by 13 Hz under stress, whereas the variation across participants in stress-induced vocal changes more than doubled these averages (Table 1). Thus, other studies examining stress under speech could have failed to find a main effect if the average voice change observed across the entire sample was too small. This is particularly probable in studies artificially inducing stress in the lab, as mean increases in voice pitch are several times smaller in the lab than in natural scenarios (Brenner et al., 1985; Mendoza and Carballo, 1998), and indeed, most studies failing to replicate stress-induced vocal changes are laboratory-based (Kirchhübel et al., 2011; Ruiz et al., 1996).

Our results underscore the importance of controlling for individual differences in the stress response, particularly for artificially induced stress, wherein salivary cortisol assays offer one reliable (Dickerson and Kemeny, 2004) and increasingly accessible and affordable (Gröschl, 2008) method for quantifying psychological stress. Nevertheless, future studies may examine whether other measures of stress predict individual differences in vocal changes and could be used as alternative controls. These may include electrocardiographic and respiratory measures, blood pressure, heart rate, or galvanic skin response (Beatty and Behnke, 1991; Johannes et al., 2000). Self-reported stress does not appear to map closely onto physiological measures of stress (Kudielka et al., 2004a; Weinberger et al., 1979). Future studies may also test whether the ability of listeners or machine learning algorithms to accurately discriminate stressed speech is mediated by the level of stress experienced by the speaker.

Considerable variation in people's responses to various stressors has been well documented, and has been tied to factors such as sex, smoking habits, various illnesses, steroid use, and the use of hormonal contraception among women (Bouma et al., 2009; Kudielka et al., 2007; Kudielka and Kirschbaum, 2005; Liening et al., 2010). Despite controlling for these variables in our study, we still observed considerable variation in the stress response. Among the women in our study, the use of hormonal contraception did not affect the cortisol response to stress. This is in contrast to some previous work that, while reporting no effect of menstrual cycle phase on cortisol levels, found that women taking hormonal contraceptives showed a blunted cortisol stress response (Bouma et al., 2009; Liening et al., 2010). Several studies also show a greater increase in free cortisol levels among men than women in response to psychosocial stress (reviewed in Kudielka and Kirschbaum, 2005), therefore the present study warrants replication among men. Our results also suggest that the effects of stress on nonverbal vocal parameters such as voice pitch can be measured from either spontaneous or read speech. However, this may not apply to analysis of verbal parameters, such as articulatory and prosodic patterns in stressed speech (see e.g., Hecker et al., 1968; Ruiz et al., 1996; Tolkmitt and Scherer, 1986).

There are many important, practical applications for research on speech under stress. Stressed speech often causes voice recognition algorithms to fail (Ververidis and Kotropoulos, 2006). The capacity to control for variation in speech under stress will therefore increase the extent to which voice recognition and activation technology can be effectively utilized (Hansen, 1996). This research also offers the possibility of accurately detecting stress from the voice signal alone, a vital tool when monitoring the psychological states of pilots, astronauts, military personnel, long-haul drivers, medical professionals, or for forensic analysis and criminal profiling (Giddens et al., 2013). However, in order to identify reliable vocal markers of stress, it



will be necessary to first remedy the problems of defining stress, standardizing methodologies used to elicit or measure stress, and controlling for individual differences in the stress response.

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## **Appendix**

Excerpt of the Rainbow Passage (Fairbanks, 1960) (Polish translation):

“Gdy słońce odbija się o krople deszczu w powietrzu, działają one jak pryzmat i tworzą tęczę. Tęcza to podział białego światła na wiele pięknych kolorów. Przyjmują kształt okrągłego łuku, który biegnie wysoko, a jego dwa końce chowają się widocznie poza horyzont.”